ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2006, p. 1170–1177 Vol. 50, No. 4
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.4.1170–1177.2006

Determinants of Rifampin, Isoniazid, Pyrazinamide, and Ethambutol Pharmacokinetics in a Cohort of Tuberculosis Patients

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Received 7 June 2005/Returned for modification 21 August 2005/Accepted 9 January 2006

Evaluation of sources of pharmacokinetic variation can facilitate optimization of tuberculosis treatment regimens by identification of avoidable sources of variation and of risk factors for low or high drug concentrations in patients. Our objective was to describe the pharmacokinetics of rifampin, isoniazid, pyrazinamide, and ethambutol in a cohort of tuberculosis patients established on first-line treatment regimens and to evaluate the determinants of pharmacokinetic variation. Plasma concentration-time profiles were determined for each of the drugs in 142 patients with drug-sensitive pulmonary tuberculosis after 2 months of daily treatment in hospital. Pharmacokinetic measures were described by noncompartmental analysis. Multiple linear regression was used to evaluate the patient and the treatment factors associated with variation of the area under the concentration-time curve from 0 to 8 h. Several factors independently associated with variations in antituberculosis drug concentrations were identified: human immunodeficiency virus infection was associated with 39% and 27% reductions for rifampin and ethambutol, respectively; formulation factors were determinants of rifampin and isoniazid bioavailability; female patients had increased rifampin and isoniazid concentrations but reduced ethambutol concentrations; older patients had higher levels of isoniazid and ethambutol; patients with a history of previous antituberculosis treatment had lower ethambutol concentrations; and the dose per kilogram of body weight was associated with the concentrations of all four agents. Further studies are required to assess the implications of variations in antituberculosis drug concentrations for efficacy and safety before decisions are made to change the dosing strategy in patients at risk.

During tuberculosis treatment the complex relationship between pathogen, host, and drug exposure is poorly understood. Target therapeutic drug concentrations based on large studies with pharmacokinetic data and outcomes have not been defined in human studies. Although favorable treatment outcomes are achievable in approximately 95% of patients with pulmonary tuberculosis who receive 6-month rifampin-based regimens under optimal conditions (12), low or high drug levels may be critical where there is incomplete drug delivery, variable drug quality, different disease presentations (with pathogens in various sites and metabolic states), human immunodeficiency virus (HIV) coinfection, severe illness, comorbid disease, and malnutrition. Moreover, the possibility should be entertained that drug products of suboptimal quality may be less well absorbed in patients with more severe disease or malnutrition. For these reasons it is important to identify (and, where possible, to limit) factors associated with pharmacokinetic variability in patient populations.

While several potential determinants of drug concentration variability are recognized (13, 15, 19, 20, 28), they are poorly characterized in tuberculosis patient populations. Low antituberculosis drug concentrations in HIV-infected patients have been reported (2, 6, 7, 17, 19, 22, 29). However, other studies do not support the association (4, 10, 27), and low drug concentrations are also described in the absence of HIV infection (4, 13, 20). Limited evidence suggests that antituberculosis drug concentrations in patients might in some circumstances be related to alcohol use (13), undernutrition (20), gender (21, 28), or drug formulation (13, 15, 28).

In this study the plasma concentrations of the first-line antituberculosis agents were studied in a large number of hospitalized tuberculosis patients from the Boland-Overberg region in the Western Cape, South Africa. Multivariate analyses were used to identify patient- and treatment-related sources of pharmacokinetic variation. Some interim findings for rifampin in a subgroup of the patients were published previously (15).

MATERIALS AND METHODS

A prospective pharmacokinetic study was conducted among 142 patients with pulmonary tuberculosis at the regional tuberculosis hospital. The study protocol was approved by the University of Cape Town Research Ethics Committee and by the regional health authorities. All participants gave written consent before inclusion. Patients had been referred to the hospital for reasons that included a poor response to treatment, suspected nonadherence, debility, severe or complicated disease, and poor socioeconomic circumstances. The daily ingestion of antituberculosis treatment was observed by the hospital staff. Two months after admission, covariate factors were recorded and the plasma concentration-time profiles of the drugs were determined. Drug doses, based on patient body weight, were those prescribed by the attending physician and are summarized in Table 1. The drug products used were those routinely administered in the hospital, and with the exception of certain batches of rifampin capsules (detailed below), all the formulations were approved for use in the country by the Medicines Control Council of South Africa.

Pharmacokinetics. The antituberculosis drugs were administered under fasting conditions, and drug administration was carefully observed by an investigator. Blood samples were obtained immediately before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 h after drug ingestion. The samples were immediately placed on ice before centrifugation (750 × *g* for 10 min) at room temperature within 30 min of collection. Plasma samples of at least 1.2 ml were stored in polypropylene tubes

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TABLE 1. Patient and treatment characteristics for study participants

Characteristic	No. of patients tested	Proportion(s) (no. [%] of patients)	Median	25th to 75th centile
Patient factors				
Demographic and clinical characteristics				
Sex	142	78 females (55%); 64 males (45%)		
New treatment or retreatment	142	51 new (36%); 91 retreatment (64%; previously treated for tuberculosis or received ≥1 mo of treatment prior to admission)		
Acetylator type	93	17 slow (18%); 76 intermediate or rapid (82%)		
HIV infection	141	14 (10%)		
Age (yr)	141		36	28–45
Chemistry[a]				
Albumin (g/liter)	142		34	31–37
ALT (units/liter)	142		14	10–19
AST (units/liter)	142		17	14–22
AP (units/liter)	141		68	58–81
γ-GT (units/liter)	142		29	19–47
Total bilirubin (μmol/liter)	142		6	4–7
Drug factors				
Dose/kg[b]				
Rifampin (mg/kg)	139		10.9	8.8–14.2
Isoniazid (mg/kg)	139		6.5	4.8–8.8
Pyrazinamide (mg/kg)	139		35.7	25.2–47.3
Ethambutol (mg/kg)	131		24.5	16.8–32.6
Formulation				
Single or FDC rifampin and isoniazid products	138[c]	29 (21%) FDCs, 109 (79%) single drug products		

[a] ALT, alanine transaminase; AST, aspartate transaminase; AP, alkaline phosphatase.
[b] The weight was not known for three subjects.
[c] The formulation type was not known for four subjects.

on dry ice until they were transferred to a −80°C freezer for storage until analysis. Published high-performance liquid chromatography methods with UV detection (25) were used to determine the plasma concentrations of rifampin, isoniazid, and pyrazinamide; and a published mass spectrometry method (5), with modifications, was used to measure the ethambutol levels in plasma. The methods were validated over the concentration ranges of 0.3 to 25 mg/liter, 0.2 to 15 mg/liter, 0.2 to 70 mg/liter, and 0.1 to 10 mg/liter for rifampin, isoniazid, pyrazinamide, and ethambutol, respectively. The proportions of the drugs recovered were 110% for rifampin, 70% for isoniazid, 95% for pyrazinamide, and 90% for ethambutol. Within- and between-day precisions were less than 15%. Drug concentrations below the limit of detection were assumed to be zero. Detectable concentrations less than the lower limit of the validated ranges were treated as missing data.

Noncompartmental analysis with WinNonlin version 3.3 (Pharsight Corp., Mountain View, CA) was used to compute the peak drug concentration (C_{max}), the time to C_{max} (T_{max}), the plasma half-life ($t_{1/2}$), the area under the curve until the last measurable concentration (AUC_{0-8}), and the area under the curve extrapolated to infinity ($AUC_{0-\infty}$).

Covariates. The patient factors taken into account included age, sex, treatment category ("new" or "retreatment"), the drug dose/kg of body weight, biochemical markers of liver function (serum alanine transaminase, serum aspartate transaminase, alkaline phosphatase, gamma-glutamyltransferase [γ-GT], and total bilirubin), the serum albumin level, and HIV infection status. The acetylator genotype and phenotype were determined for a subgroup of 93 patients by using published methods, with minor modifications (8, 16). The product details that were noted included whether fixed-dose combination (FDCs) products containing rifampin and isoniazid or single drug products were administered. All participants received single drug products of pyrazinamide and ethambutol.

Statistics. Multivariate linear regression analysis was used to determine the patient and the drug factors associated with the AUC_{0-8}. Variable selection was initially by an automated backwards stepwise process (variables with P values ≥0.055 were removed from the model and were added if the P value was <0.050), followed by a forward stepwise procedure, based on the contribution of individual variables to the overall fit of the model (variables with P values <0.050 were added). The model assumptions of constant variance, linearity, and the appropriate form of the covariates in the model were checked by using methods based on the distribution of the residuals. Univariate linear regression analyses were used to report the unadjusted associations of each independent variable included in the models. Stata version 8.2 (Stata Corp., College Station, TX) was used to compute summary statistics and for statistical modeling.

RESULTS

The patient and treatment covariates evaluated are summarized in Table 1. The prevalence of HIV infection among the study cohort was 10%. None of the patients complained of symptomatic diarrhea, although the symptom was not specifically sought. The vast majority of patients were ambulant at the time of evaluation. Acetylator type was dichotomized into slow or intermediate plus rapid. Generally, there was agreement between the phenotype and the genotype. The genotype was used for four patients for whom there was discordance between the genotype and phenotype. The phenotype was used for six subjects for whom acetylator genotype determination was not successful. The concomitant medications used by the study population are shown in Table 2; they were not expected to be an important source of variation in the antituberculosis drug levels. As all patients received rifampin, isoniazid, and pyrazinamide, it was not possible to evaluate the drug-drug interactions between these drugs. Ethambutol use was contraindicated in 10 patients; the concentrations of rifampin, isoniazid, and pyrazinamide were not significantly different between these patients and those receiving ethambutol.

TABLE 2. Concomitant medications used by the study population

Concomitant drug	No. of patients
Amiloride-hydrochlorothiazide combination	2
Chlorpromazine	1
Co-trimoxazole	2
Digoxin	1
Ethionamide	2
Ferrous sulfate	2
Fluphenazine	1
Folic acid	4
Furosemide	1
Glibenclamide	1
Gliclazide	2
Insulin	3
Magnesium trisilicate	1
Orphenadrine	1
Methyldopa	2
Perindopril	3
Phenobarbitone	1
Phenytoin	2
Pholcodeine	3
Piroxicam	2
Potassium chloride	1
Prednisone	2
Pyridoxine	124
Ramipril	1
Streptomycin	68
Vitamin B complex	51
Warfarin	1

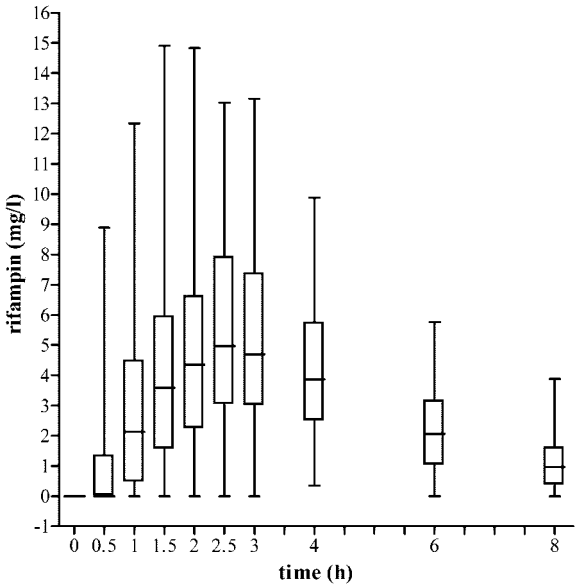

FIG. 1. Median concentration of rifampin at each sampling time for participants who received approved products ($n = 88$). Error bars indicate the ranges of concentrations at each sampling time, and the boxes represent the 25% to 75% percentile ranges.

Rifampin. Five brand name rifampin-containing formulations were used by the participants on the day of pharmacokinetic assessment {30 patients received Rifacap 150 [Lupin Laboratories Ltd.] and 42 patients received Rifacap 450 [Lupin Laboratories Ltd.]; 37 patients received Rimactane 600 [Rolab (Pty.) Ltd.]; and 29 patients received FDCs of rifampin and isoniazid; of these 29 patients, 26 received Rifinah 150 [Aventis Pharma (Pty.) Ltd.] and 3 received Rifinah 300 [Aventis Pharma (Pty.) Ltd.]}. For four participants, the formulation details were not recorded. Fifty-four (38%) of the patients studied received batches of rifampin capsules which were subsequently withdrawn by the national medicines regulatory authority on the grounds that insufficient bioavailability data were submitted after what the manufacturer had considered to be minor formulation changes (15). Twelve patients who received the formulation Rifacap 150 and all those who received Rifacap 450 were administered these nonapproved batches. The pharmacokinetic measures for the nonapproved and the approved product batches are summarized in Table 3. Because the rifampin levels achieved in patients who received the nonapproved products were significantly different from those in patients who received products that met the regulatory requirements (15), only the data from the latter group are presented further.

Wide variations in the plasma concentrations of rifampin were demonstrated (Fig. 1). Low levels were common; 61 of the 88 participants (69%) who received approved products had C_{max}s below the reference range of 8 to 24 mg/liter (18), and 19 (22%) had very low peak concentrations (<4 mg/liter). The data were insufficient to determine the AUC_{0-8} for one participant and the $t_{1/2}$ and the $AUC_{0-\infty}$ for five participants.

The multiple linear regression model (shown in Table 4) described 36% of the variability associated with AUC_{0-8}. Reductions of 8.69 mg · h/liter ($P = 0.001$), 8.37 mg · h/liter ($P = 0.004$), and 8.34 mg · h/liter ($P = 0.051$) were demonstrated in

TABLE 3. Summary of the pharmacokinetic measures for rifampicin, by formulation regulatory status

Batch and parameter	2-h level (mg · liter^{-1})	T_{max} (h)	C_{max} (mg · liter^{-1})	$t_{1/2}$ (h)	AUC_{0-8} (mg · h · liter^{-1})	$AUC_{0-\infty}$ (mg · h · liter^{-1})
Approved batches						
No. of patients	88	88	88	83	87	83
Median	4.4	2.5	5.9	1.9	21.5	25.6
Interquartile range	2.1–6.7	2.0–3.0	4.2–8.4	1.5–2.5	15.3–31.7	16.6–36.0
Minimum–maximum	0–14.8	1.0–8.0	1.3–14.9	0.9–4.7	3.6–65.8	5.6–80.1
Nonapproved batches						
No. of patients	54	49	54	42	54	42
Median	2.1	2.5	3.8	1.8	13.7	18.9
Interquartile range	0.0–5.3	2.0–3.0	1.3–6.7	1.6–2.3	4.6–26.0	12.1–31.4
Minimum–maximum	0.0–20.4	1.0–8.1	0.0–20.4	1.0–6.8	0.0–81.6	4.0–102.7

male patients, those who received the FDC products, and HIV-infected individuals, respectively. Each dose increment of 1 mg/kg resulted in an AUC_{0-8} increase of 2.51 mg · h/liter ($P = 0.003$), and each 1-μmol/liter increase in the total serum bilirubin concentration was associated with an increment of 0.655 mg · h/liter ($P = 0.034$) in the AUC_{0-8}. Six observations were excluded due to missing covariate data. As univariate analysis did not support the association with HIV infection, indicating that adjustment for the other risk factors was necessary to demonstrate the effect of HIV infection in this small sample (nine participants who received approved products had HIV infection), this finding should be interpreted with caution. The univariate analyses for sex ($P = 0.043$), formulation type ($P = 0.076$), dose per kilogram of body weight ($P = 0.002$), and total serum bilirubin concentration ($P = 0.007$) supported the findings of the multivariate analysis.

Isoniazid. The brand name isoniazid-containing products used included Lennon-Isoniazid (200-mg tablet; Pharmacare Ltd.) together with Be-tabs Isoniazid (100 mg tablet; Be-tabs Pharmaceuticals [Pty] Ltd.) in 21% of the patients, Norstan-Isoniazid (200-mg tablet; Norstan Ltd.) with Be-tabs Isoniazid in 14% of the patients, and Be-tabs Isoniazid alone in 43% of the patients. For 29 patients FDC products of rifampin and isoniazid were used.

The median isoniazid concentrations are shown in Fig. 2; the pharmacokinetic measures derived by noncompartmental analysis are displayed in Table 5. For one participant, incomplete data prevented characterization of the AUC and the $t_{1/2}$. Only 3 of the 142 participants (2%) had peak concentrations less than 3 mg/liter (the lower limit of the reference range [18]).

Multivariate regression analysis (presented in Table 4) explained 27% of the variability associated with isoniazid AUC_{0-8} values in the study cohort. Patients taking FDCs and

FIG. 2. Median concentrations of isoniazid at each sampling time ($n = 142$). Error bars indicate the ranges of concentrations at each sampling time, and the boxes represent the 25% to 75% percentile ranges.

TABLE 4. Multiple linear regression models describing the AUC_{0-8} for rifampin, isoniazid, pyrazinamide, and ethambutol and β coefficients (95% confidence intervals) for associations between AUC_{0-8} and the covariate factors in univariate linear regression analyses

Parameter	β Coefficient (95% confidence interval) describing the AUC_{0-8}s for the following drugs with the indicated regression model[a]:							
	Rifampin (21.5 [15.3–31.7])[e]		Isoniazid (25.0 [18.9–32.9])		Pyrazinamide (288.4 [245.7–335.4])		Ethambutol (19.9 [16.3–24.2])	
	Multivariate ($n^b = 81$)	Univariate	Multivariate ($n^b = 133$)	Univariate	Multivariate ($n^b = 137$)	Univariate	Multivariate ($n^b = 125$)	Univariate
Male sex	−8.7 (−13.7 to −3.7)	−5.9 (−11.6 to −0.2)	−5.7 (−8.9 to −2.5)	−4.6 (−8.2 to −1.0)	NS		+2.4 (0.4 to 4.5)	+2.1 (−0.1 to 4.4)
Age (yr)	NS[c]		+0.1 (0.0 to 0.3)	+0.2 (0.0 to 0.3)	NS		+0.1 (0.0 to 0.2)	+0.1 (0.0 to 0.2)
Retreatment	NS		NS		NS		−3.5 (−5.8 to −1.3)	−1.6 (−4.0 to 0.9)
HIV infection	−8.3 (−16.7 to 0.0)	−3.6 (−13.1 to 6.0)			NS		−5.5 (−9.1 to −1.9)	−4.5 (−8.3 to −0.7)
FDC formulation	−8.4 (−13.9 to −2.8)	−5.6 (−11.8 to 0.6)	−6.9 (−10.7 to −3.1)	−5.5 (−10.0 to −1.0)	—[d]		—	
Dose (mg/kg)	+2.5 (0.9 to 4.1)	+2.9 (1.2 to 4.7)	+1.5 (0.2 to 2.7)	+1.8 (0.4 to 3.2)	+7.5 (5.8 to 9.1)	+7.8 (6.0 to 9.6)	+0.4 (0.1 to 0.6)	0.4 (0.2 to 0.7)
Albumin level (g/liter)	NS		NS		NS		−0.3 (−0.5 to −0.1)	−0.4 (−0.6 to −0.2)
Bilirubin level (μmol/liter)	+0.7 (0.1 to 1.3)	+1.0 (0.3 to 1.7)	+0.1 (0.0 to 0.1)	+0.1 (0.0 to 0.1)	+3.6 (1.1 to 6.1)	+3.8 (0.5 to 7.0)	NS	
γ-GT (units/liter)	NS		NS		NS		NS	

[a] The changes in AUC_{0-8} shown are associated with unitary increases in the covariate factors. Multivariate analyses showed no significant relationship of alanine transaminase, aspartate transaminase, or alkaline phosphatase and AUC_{0-8} for any drug.

[b] n, the number of observations included in the model; unless indicated otherwise in the text, observations were dropped from the model because significant covariates were missing.

[c] NS, no significant relationship with AUC_{0-8} in multivariate model.

[d] Single drug products were used in all participants.

[e] Data in parentheses represent the median (interquartile range) AUC_{0-8} in milligrams · hour · liter^{-1}.

TABLE 5. Summary of pharmacokinetic measures for isoniazid, pyrazinamide, and ethambutol

Drug and parameter	2-h level ($mg \cdot liter^{-1}$)	T_{max} (h)	C_{max} ($mg \cdot liter^{-1}$)	$t_{1/2}$ (h)	AUC_{0-8} ($mg \cdot h \cdot liter^{-1}$)	$AUC_{0-\infty}$ ($mg \cdot h \cdot liter^{-1}$)
Isoniazid						
No. of patients	142	142	142	141	141	141
Median	5.0	1.5	6.5	2.8	25.0	32.5
Interquartile range	3.6–7.1	1.0–2.5	4.9–8.7	2.1–3.5	18.9–32.8	22.5–42.4
Minimum–maximum	0.4–13.6	0.5–4.0	0.5–15.0	1.0–8.4	1.7–64.0	3.5–87.2
Pyrazinamide						
No. of patients	142	142	142	141	141	141
Median	49.6	2.0	52.7	5.9	288.4	499.7
Interquartile range	41.1–56.4	1.1–2.5	46.0–61.4	4.9–7.1	245.7–335.4	406.2–632.3
Minimum–maximum	1.3–86.6	0.5–4.1	1.5–91.8	2.7–12.9	9.0–510.2	18.7–1,246.8
Ethambutol						
No. of patients	127	129	129	123	129	123
Median	3.1	3.0	5.0	2.6	19.9	24.9
Interquartile range	2.2–4.4	2.0–3.4	4.1–6.3	2.1–3.1	16.3–24.2	19.2–30.8
Minimum–maximum	0.8–9.8	1.0–6.1	0.2–10.4	1.1–8.1	0.2–45.2	6.9–54.0

male patients had substantial AUC_{0-8} reductions of 6.90 $mg \cdot h \cdot liter^{-1}$ ($P = 0.001$) and 5.68 $mg \cdot h \cdot liter^{-1}$ ($P = 0.001$), respectively. For each 1-mg/kg increase in the weight-adjusted dose, each year of age, and each 1-unit/liter γ-GT increase, increments of 1.50 $mg \cdot h \cdot liter^{-1}$ ($P = 0.019$), 0.14 $mg \cdot h \cdot liter^{-1}$ ($P = 0.037$), and 0.05 $mg \cdot h \cdot liter^{-1}$ ($P = 0.007$) in the AUC_{0-8} were found, respectively. One outlying and influential observation was excluded in order to satisfy the mathematical (constant variance) assumptions of the model. As all participants in whom acetylator status was characterized received products containing single drugs, a second multivariate regression model (data not shown) described the covariate effects in this group: the rapid and intermediate acetylators had AUC_{0-8} values 6.73 $mg \cdot h \cdot liter^{-1}$ ($P = 0.006$) lower than those of the slow acetylators. The relationships of the other covariates to AUC_{0-8} were similar for the two models and were supported by univariate analyses demonstrating significant associations at the level of 0.07.

Pyrazinamide. The brand name pyrazinamide formulations used included Rolab-Pyrazinamide (500-mg tablet; Rolab (Pty.) Ltd.) in 34% of the patients, Pyrazide (500-mg tablet; Rolab (Pty.) Ltd.) in 42% of the patients, Rozide (500-mg tablet; Rolab (Pty.) Ltd.) in 7% of the patients, and Isopas (500-mg tablet; Pharmacare Ltd.) in 17% of the patients.

The pyrazinamide concentrations in the study cohort (summarized in Table 5 and Fig. 3) displayed less variability than the concentrations of the other drugs. For one participant, the data were insufficient to determine the AUC and the $t_{1/2}$ values. Only one subject had a C_{max} of less than 20 mg/liter (the lower limit of the reference range [18]).

Multivariate regression (Table 4) found that only two covariates had significant associations with the AUC_{0-8}; they explained 40% of the variability. Each increment of 1 mg/kg in the weight-adjusted dose and each 1-μmol/liter increase in the bilirubin level were associated with respective increases of 7.48 $mg \cdot h \cdot liter^{-1}$ ($P = 0.000$) and 3.60 $mg \cdot h \cdot liter^{-1}$ ($P = 0.004$) in the AUC_{0-8}. One outlying and influential observation was excluded. The associations were supported by the univariate analyses, which were significant at the level of 0.05.

Ethambutol. The brand name products containing ethambutol included Purderal (400-mg tablet; Pharmacare Ltd.) in 23% of the patients and Rolab-Ethambutol (400 mg tablet; Rolab (Pty.) Ltd.) in 77% of the patients.

The pharmacokinetics of ethambutol in the study cohort are summarized in Table 5 and Fig. 4. The $t_{1/2}$ and $AUC_{0-\infty}$ values for two observations were excluded due to a poor goodness of fit of the elimination rate constant. Plasma samples for 3 participants were lost prior to ethambutol concentration determination, and 10 participants were not prescribed ethambutol. Three (2%) participants had peak ethambutol concentrations less than 2 mg/liter (the lower limit of the reference range [18]).

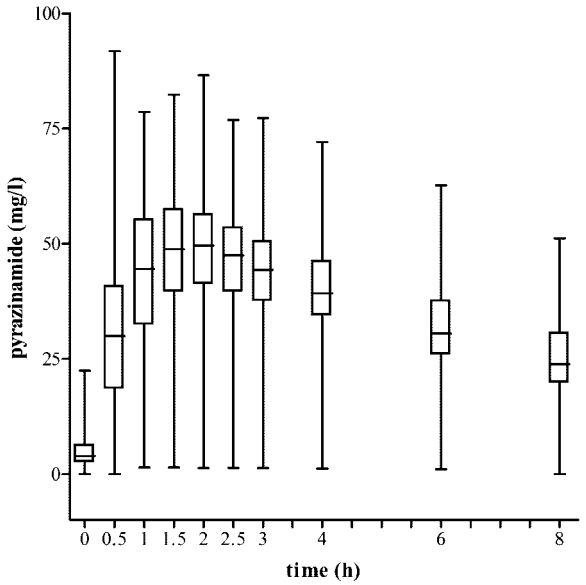

FIG. 3. Median concentrations of pyrazinamide at each sampling time ($n = 142$). Error bars indicate the ranges of concentrations at each sampling time, and the boxes represent the 25% to 75% percentile ranges.

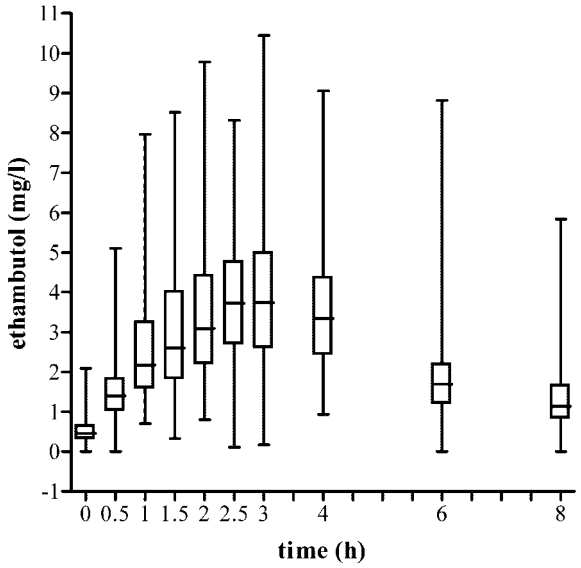

FIG. 4. Median concentrations of ethambutol at each sampling time ($n = 129$). Error bars indicate the ranges of concentrations at each sampling time, and the boxes represent the 25% to 75% percentile ranges.

Multivariate linear regression (Table 4) explained 31% of the variability associated with AUC_{0-8}. HIV infection was associated with the most substantial reductions (5.48 mg · h · liter^{-1}; $P = 0.003$). Retreatment patients had lower ethambutol AUC_{0-8} values (−3.52 mg · h · liter^{-1}; $P = 0.002$) than patients receiving treatment for the first time. In contrast to the findings for isoniazid, female patients had lower ethambutol AUC_{0-8} values (−2.45 mg · h · liter^{-1}; $P = 0.018$) than male patients. Patients receiving higher weight-adjusted doses and older patients had increased ethambutol AUC_{0-8} values (0.35 mg · h · liter^{-1}for each 1 mg/kg [$P = 0.004$] and 0.11 mg · h · liter^{-1} for each year [$P = 0.012$]), and higher albumin levels were associated with AUC_{0-8} reductions (−0.30 mg · h · liter^{-1} for each 1 g/liter; $P = 0.003$). The associations were supported by the univariate analyses, which were significant at the 0.05 level, apart from sex ($P = 0.066$) and treatment category ($P = 0.200$).

DISCUSSION

In this study we have described the plasma concentrations of rifampin, isoniazid, pyrazinamide, and ethambutol in a cohort of hospitalized tuberculosis patients and identified patient- and treatment-related sources of pharmacokinetic variation. Although less than 10% of the patients treated for tuberculosis in the province are hospitalized, they comprise an estimated 4,000 annually.

The prevalence of very low rifampin levels is reason for concern. Although the therapeutic relationship between rifampin concentrations and treatment response has not been defined in human studies, higher doses are associated with improved early bactericidal activity and better treatment results (14, 24). A mouse model suggests that the drug's activity is concentration dependent and is related to the AUC/MIC ratio (11). If a rifampin MIC of 1 mg/liter for *Mycobacterium tuberculosis* (which allows protein binding in vivo [11]) is assumed, the median ratio of the $AUC_{0-\infty}$/MIC in this study was 25.62 for patients who received approved products. This is severalfold lower than the estimated levels required for optimal efficacy (11). Furthermore, in keeping with the findings of several other studies with tuberculosis patients (4, 7, 20, 26, 28), low peak concentrations in comparison to the published reference range (18) were demonstrated in the majority of patients. Autoinduction of rifampin's metabolism is expected to result in lower levels after repeated doses (9, 24), and this might explain in part the relatively low concentrations in the patient studies. However, other factors may be important, as the levels reported differ between patient populations (26).

The majority of patients achieved levels of isoniazid, pyrazinamide, and ethambutol within or above the expected ranges. This contrasts with the findings of two African studies: Choudri et al. demonstrated peak isoniazid levels <3 mg/liter in 89% of patients (4), and Tappero et al. found that low concentrations of isoniazid and ethambutol were common (26). Furthermore, Peloquin et al. (19) showed that substantial proportions of patients with HIV infection had isoniazid and ethambutol levels below the recommended ranges, and Zhu et al. found that ethambutol levels <2 mg/liter occurred frequently (29). Differences in patient characteristics, dosing practices, and methods of pharmacokinetic evaluation may account for the discrepancies. One participant in our study who had very low levels of all four drugs (C_{max} values for rifampin, isoniazid, pyrazinamide, and ethambutol were 0 mg/liter, 0.49 mg/liter, 1.47 mg/liter, and 0.16 mg/liter, respectively) and from whose sputum drug-sensitive organisms were recurrently isolated after 5 months of treatment may represent an important minority of patients at high risk of treatment failure. Interestingly, she had few of the risk factors for low drug concentrations identified in this study.

Important differences in the rifampin and isoniazid concentrations were achieved between patients who received single drug formulations and those who received FDC products. Although insufficient single drug and FDC products were represented in the study to confirm whether the finding of lower concentrations in the FDCs can be generalized, it indicates that differences between pharmaceutical products have a marked effect on the bioavailability of the drugs in patients. The FDC products used by the patients in this study had undergone and passed in vivo bioequivalence testing in studies with healthy volunteers before their registration approval by the national regulatory authority and were used well before their expiry dates (with a median time to expiry of 39 months). Questions therefore arise about the effectiveness of bioequivalence testing prior to product registration, ongoing quality assurance procedures for the monitoring of subsequent batches, and the storage conditions of products prior to their use (23).

HIV infection was an important determinant of the concentrations of rifampin and ethambutol. Although there are several other reports of low rifampin levels in patients with HIV infection, the 39% reduction in the AUC_{0-8} for rifampin associated with HIV infection should be confirmed in a larger study. The 27% reduction in the AUC_{0-8} for ethambutol in HIV-infected patients was similar to that observed by Zhu et al. (29). None of the HIV-infected patients had diarrhea, and

only four had $CD4^+$ lymphocyte counts less than 200/μl; it is possible that the reductions in isoniazid levels reported in HIV-infected patients in other studies could be attributed to diarrhea (7, 22). Female patients had higher rifampin and isoniazid concentrations but lower ethambutol concentrations than male patients. Although the mechanisms of the sex-related differences in drug concentrations are poorly understood, they have been observed for several drugs, and the findings for isoniazid are consistent with those of other investigations (21). The higher isoniazid and ethambutol levels in older patients are not surprising, as the reduced activity of metabolic and excretory pathways is expected with aging. When adjusted for other important risk factors, a history of previous tuberculosis was associated with lower ethambutol concentrations. The association was not significant in the univariate analysis ($P = 0.200$) and needs to be confirmed by further studies, as it might reflect a propensity for tuberculosis to relapse in patients who have low ethambutol concentrations. The association of bilirubin and rifampin levels is consistent with their competition for biliary elimination (1). The relationship of total bilirubin with rifampin and pyrazinamide levels might also reflect a reduced hepatic capacity to clear bilirubin and the two drugs. These findings and the association of the serum γ-GT level with isoniazid concentrations are of little clinical importance, as the magnitudes of the effects are small and the majority of patients had markers of hepatic function within the normal ranges. Patients with reduced serum albumin concentrations had higher ethambutol levels. The reason for this is unclear, but it is possible that altered pharmacokinetics in patients with more severe disease or malnutrition is responsible. The relationship of the concentrations of all four drugs to the dose per kilogram of body weight supports the widely used strategy of using weight groups to guide dosing practice.

Weaknesses of the study include incomplete data for some participants, the failure to assess the variability of the pharmacokinetics within patients, and the pharmacokinetic evaluation of only one time point during treatment. Furthermore, decisions about admission to the study facility, for example, for reasons such as a poor response to treatment, may have biased the study to enrolling patients more likely to have abnormal pharmacokinetics. Lastly, treatment response was not assessed in a uniform manner that might allow insight into the consequences of the pharmacokinetic variability.

In conclusion, substantial variability of antituberculosis drug concentrations was demonstrated among a cohort of patients. Several risk factors for drug concentration variation were identified. Much of the variability remains unexplained. Further studies are needed to verify the findings with other patient populations, to identify further sources of variation, and to determine optimal dosing strategies. In particular, it is necessary to define that component that may be attributed to intra-individual variation in order to assess the adequacy of using drug concentration measurement during a single dosing interval to predict drug exposure for the duration of treatment. As the pharmacokinetic consequences of antituberculosis drugs remain to be defined, further studies are required before rational decisions can be made as to changes in dosing in patients at risk of low or high drug concentrations. The low concentrations of rifampin found in many patients are cause for concern. A minority of patients had very low concentrations of rifampin, isoniazid, pyrazinamide, or ethambutol; this supports previous recommendations that drug concentration measurement is necessary in patients with an inadequate response to directly observed therapy (3, 13).

ACKNOWLEDGMENTS

This research was funded by the Division of Pharmacology, University of Cape Town, and the Medical Research Council of South Africa.

We thank the staff and patients of Brewelskloof Hospital, staff members of the Division of Clinical Pharmacology of the University of Cape Town, and Francesca Little for their contributions to the conduct and analysis of the research.

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